TAXON 23(2/3): 271-276. MAY 1974

PROTISTAN PHYLOGENY: MULTIPLE WORKING HYPOTHESES*

*Helen Tappan***

Summary

The method of the "multiple working hypothesis" is best applied to "those subjects in which, while much is known, more remains to be known," a description which certainly is applicable to the Phylogeny of the Protista. Although various proposed phylogenies have been stoutly defended by their authors as unassailable, each has been based on one or another line of evidence, generalization or previous assumption which is contested strongly by others.

Previous workers have regarded the Protista as animals or as plants, as one distinct kingdom, or as including representatives of two, three or as many as 13 kingdoms. Classifications and phylogenetic interpretations have been based on various aspects of morphology, cytology, ultrastructure, biochemistry, physiology, life cycles, or the fossil record. Each resulting classification has much supporting evidence but leaves unanswered other vexing problems.

The present symposium includes only a sampling of the types of approach currently being used, but may aid in stimulating additional interest and study of the highly diverse and intriguing organisms known as the Protista.

More than four score years ago, the noted geologist Thomas C. Chamberlin formulated a method of "multiple working hypotheses" for creative study, which he published in Science (reprinted, Chamberlin, 1965). He suggested that its application lay best "in those subjects in which, while much is known, more remains to be known." He observed that "the moment one has offered an original explanation for a phenomenon which seems satisfactory . . . affection for his intellectual child springs into existence . . ." There is then a tendency to search for and emphasize favorable facts, together with a bias toward neglect or even suppression of facts that do not coincide, until eventually a prematurely advanced explanation becomes a "ruling theory". To avoid this pitfall, Chamberlin advocated the method of the multiple working hypothesis, in which the investigator becomes "parent of a family of hypotheses . . ." and hopefully would not then unduly favor one to the detriment of another. The method was regarded as particularly valuable where, as in many instances, an adequate explanation in fact involves the coordination of several agencies in varying proportions, whereas emphasis on a single hypothesis would ignore or suppress such possible multiple explanations.

As it appears probable that no one individual could be sufficiently well

* This paper was the first of a series of eight presented at the First International Congress of Systematic and Evolutionary Biology, Boulder, Colorado, USA, 10 August 1973 as Symposium no. 10 entitled *The Phylogeny of Protista*. The first three papers are published in this issue of Taxon (Tappan, Loeblich, De Ley); the second series (Fott, Grambast, Jahn et al., Corliss) will be published in the issue for August 1974. The paper by Manton could alas not be published due to technical reasons.

** Department of Geology, University of California at Los Angeles, Los Angeles, California, 90024, U.S.A.

versed to compile, evaluate and utilize all the biochemical, statistical, morphological, ultrastructural, physiologic, ontogenetic, and paleontological information available for the bacteria, blue-green, green, red, brown, and yellow-brown algae, protozoa and fungi, in order to singly formulate "multiple working hypotheses" for the phylogeny of the protista, the best alternative would appear to lie in a thorough consideration of the poly-parental available hypotheses to determine whether one or another, a combination of many, or even other as yet unproposed explanations, best fits the already accumulated and the yet-to-be-discovered data.

To begin with, different workers vary in their concept of the Protista. Some exclude the Monera (bacteria and blue-green algae), others regard these as Lower Protists. Most include in the Protista all protozoans and eukaryotic algae, but some exclude the red, green and brown multicellular algae; some include the fungi, and others exclude these.

Copeland (1956) recognized four kingdoms of organisms, the Mychota (prokaryotes), Protoctista (including the Rhodophyta, Phaeophyta s.l. in which the classes and orders of the Chrysophyceae, Bacillariophyceae, and the oomycetes were combined with the Phaeophyta s.s, the Pyrrhophyta, Fungi, Mycetozoa, Rhizopoda and flagellate protozoa, Sporozoa and Ciliophora), the Plantae (which included the algal Chlorophyta as well as the higher plants), and Animalia.

Whittaker (1959) recognized the Monera and Eunucleata (phytoflagellates and protozoans) as subkingdoms of the Protista, whereas the multicellular algae were considered as subkingdoms (Rhodophyta, Phaeophyta and Euchlorophyta) within the Kingdom Plantae. Higher plants were placed with the green algae in the Chlorophyta. The Kingdoms Animalia, Plantae and Fungi were defined on mode of nutrition, but the Kingdom Protista includes all three modes of nutrition, i.e., ingestion, photosynthesis and absorption. Somewhat later, Whittaker (1969) recognized five kingdoms, separating the Monera as distinct from the eukaryotic Protista.

In still another variation, Dougherty & Allen (1960) include within the Protista, the Monera (bacteria and blue-green algae), Mesoprotista (for the red algae), and Metaprotista (including everything else exept the Metaphyta and Metazoa).

In a different vein, Dillon (1963) divided all organisms into 14 kingdoms on a cytological basis; 13 of these include members that are generally included with the Protista.

Phylogeny and classification at levels below the Kingdoms also have varied considerably. Bacteria have generally been left to the microbiologists, phycologists have studied both prokaryotic and eukaryotic algae, while protozoologists also have claimed as protozoans the flagellated eukaryotic algae and the myxomycetes. Phycologists generally have emphasized the importance of the various photosynthetic pigments for classification and evolutionary reconstruction (e.g., Pascher, 1914; Tilden, 1933; Papenfuss, 1955), considering the algae to show parallel evolution in gross morphology. Thus, the single class Chlorophyceae of the Division Chlorophyta includes the flagellate Volvocales, coccoid Chlorococcales, the siphonate Siphonocladales and the Zygnematales. Other flagellated algae are variously placed in the Cryptophyceae, Dinophyceae, Chrysophyceae, and Haptophyceae of the Chromophyta (Christensen, 1966) and the Euglenophyceae and Prasinophyceae of the Chlorophyta. In contrast, the Protozoologists (Honigberg et al., 1964) place all the flagellate taxa in a single class,

Phytamastigophorea (orders Chrysomonadida, Silicoflagellida, Coccolithophorida, Heterochlorida, Cryptomonadida, Dinoflagellida, Ebriida, Euglenida, Chloromonadida, and Volvocida), and disclaim completely the coccoid, filamentous, and siphonous taxa that are aligned with these groups by the phycologists. Some phycologists regard an algal "class" as necessarily encompassing all the morphological variations (Fott, this symposium), leaving unassigned as "residual flagellates" those not similarly represented by filamentous and thallose forms.

Classically, evolution has been assumed to proceed from the bacteria through blue-green algae, to green and other eukaryotic algae. Various groups that secondarily lost their plastids (for example, some euglenids, diatoms, coccolithophorids and other apochromatic flagellates) eventually "became" protozoans and fungi. Metazoans arose from the protozoa, and metaphytes from the green algae.

In a radically different interpretation, Margulis (Sagan, 1967; Margulis, 1969) regards all eukaryotic cells as the result of a series of symbioses, one symbiont producing the (9+2) homologue as flagella, flagellar basal body, and intranuclear and extranuclear division centers, other endosymbionts becoming the mitochondria of the protozoa; eventually various endosymbiotic prokaryotic algae became the plastids of the eukaryotic algae, and of the higher plants. The monad, filamentous, and siphonate hosts that acquired similar green symbionts, for example, resulted in the morphologic variety of the Chlorophyta and are not otherwise closely related. Following in part Copeland's (1956) classification, but with five kingdoms, as in Whittaker (1969), the phylogeny of the lower eukaryotic algae was based on the homologies of the "host", and the presumed stage of protozoan development at which the symbiosis was believed to have arisen. Thus, various groups of "green algae" were regarded as of wholly independent origin, the desmids arising early with the amoeboid protozoans, the Sphaeropleacea and Oedogoniales somewhat later with the higher plant lineage, and the Euglenids and Siphonaleans arising at a more advanced "protozoan" stage of development; still later the Chlorellaceae, Volvocales and Vaucheriales branched off as they acquired green endosymbionts. The Chrysophytes similarly evolved independently at various times, and were similarly polyphyletic. The end result was not far different from Dillon's (1963) 14-kingdom proposal.

Margulis (1971) regards the prokaryote-eukaryote dichotomy of cell type to be the major basis for the symbiotic theory, bacteria being the most primitive living organisms; photosynthetic bacteria were ancestral to the blue-green algae. However, Bisset (1973) suggests that the inner layer of the bacterial cell envelope is equivalent to the nuclear membrane of the eukaryotes. Rather than being primitive, the bacteria are "highly specialized organisms, making use of their small size and consequent low bulk-surface ratio and rapid metabolism. They are almost certainly derived from other, more primitive, flagellate protista, and the origin of their organs must be sought in the general structural pool." He regards the systematic association of bacteria and blue-green algae as prokaryotes to be based on an invalid assumption.

Although protistans have at times been regarded as too simple and primitive to have shown any evolutionary tendencies, or even to have undergone any evolution at all, they nevertheless have been used by paleontologists as index fossils for stratigraphic zonation, age dating, and

correlation; for these workers, protistan evolution is an obvious and well documented fact. After all, the fossil record indicates that the protistans have been evolving for some three billion years, and the eukaryotic protists for at least a billion years; the protists thus have an evolutionary history some five times as great as any metazoan, and some 7.5 times as long as any metaphytes yet known. As has been observed many times, protistan specialization has been biochemical, cytological, morphological, and physiological, without involving compartmentalism (e.g., Dobell, 1911; Hutner & Provasoli, 1951). Protists differ from other organisms mainly in being smaller, and these size limitations affect their chemistry, metabolism, movement, and dispersal, prey-predator relationships, and even reproduction, virtually every cell being a potential reproductive cell. Their evolution thus contrasts sharply with that of the higher organisms, in which major evolutionary trends show a progressive increase in size, higher levels of structural organization and differentiation, greater efficiency of particular functions, and more elaborate nervous systems (Tax & Callender, 1960). Although supposedly geologically long-lived, detailed study of the protistan fossils shows that these represent a variety of misidentified taxa, which in fact are highly distinctive for the various geologic periods (e.g., Foraminifera, Radiolaria, Pyrrhophyta, Coccolithophorida, etc.), and their evolutionary history includes past periods of major diversification alternating with times of severe reduction, elimination, and extinction (Tappan & Loeblich, 1972).

Various phylogenetic proposals have been based on pigments and storage products, flagellar characters, nuclear phenomena, variations in cytokinesis, general morphology, and even life cycles, but, unfortunately, almost none of these can be documented among the fossil forms. No fossil protozoa are known, other than from their lorica, test or skeleton, as they lack even the cell wall of the plant protists. Based on the data available, the cytological characters cannot be shown unequivocally to be convergent or to be conservative and hence reliable in assigning relationships. As one example of the problem, the green algal Order Dasycladales are assumed to have a long geologic history (beginning in the Precambrian) with three families and some 28 tribes recognized in the family Dasycladaceae. Yet only four of these tribes have living representatives, and 93 percent of the genera and 86 percent of the tribes are extinct. The fossils generally are accepted as dasyclads on the basis of their similar morphology, with axis, whorled branches, differentiated reproductive organs, and lime encrusted surface. In view of the extensive amount of convergent and parallel evolution in the algae, could these instead be extinct fossil representatives of some other lineage (perhaps Xanthophyceae, Phaeophyceae, or Chrysophyceae)? Similarly, are the living colorless flagellates primitive, having never obtained the endosymbiont plastids, or have they secondarily lost their primitive plant-like characters? Are all the living flagellate monads more closely interrelated than are any monads to the coccoid, filamentous or thalloid forms with similar pigment complexes? Are the extant bacteria truly comparable to the primitive Precambrian organism, or are they instead degenerate and specialized protozoans? Should one expect to find living intermediates between groups that diverged a billion years ago? As the major algal diversification occurred during the Precambrian (fossil Schizophyta, Cyanophyta, Rhodophyta, Phaeophyta, and Prasinophycean and Chlorophycean Chlorophyta have been reported from rocks of this

age), this would involve both freezing in mid-step organisms at the various evolutionary levels as these occurred, while simultaneously allowing progressive evolution to continue unhindered among other populations. Do such apparent living "intermediates" represent instead an evolutionary process now underway leading to diversification in a totally different direction from that which occurred during the Precambrian?

Perhaps many questions as to the method of origin and the nature and time of protistan evolution and diversification, their phylogeny and interrelationships, must remain forever unanswered. Because these subjects are being studied intensively from many viewpoints, and our knowledge of the Protista has rapidly increased over the past decade or two, there is much cause for optimism. This Symposium on the Phylogeny of the Protista was organized for the First International Congress of Systematic and Evolutionary Biology with the aim of presenting a sampling of the varied kinds of evidence currently used for phylogenetic interpretation, differing possible methods of attacking the problems involved, and a broad scattering of the protistan taxa, as well as various levels in the taxonomic hierarchy. Its length could have been increased tenfold with almost no repetition of data, methods, or taxonomic entities and many groups were omitted from consideration, hence no consensus of opinion as to Protistan Phylogeny was sought; our multiple working hypotheses remain highly diverse, each with its own champions. Hopefully, interest in this extremely varied, previously neglected, most ancient Kingdom (or Kingdoms) of organisms has been stimulated further by this symposium and the papers here included.

Acknowledgements

The writer's research on the Protista has been supported by the Oceanography Section, National Science Foundation, NSF Grant GA-28756.

References

Bisset, K. A. 1973 – Do bacteria have a nuclear membrane? Nature 241: 45.

Chamberlin, T. C. 1965 – The method of multiple working hypotheses. Science 148: 754-759.

Christensen, T. 1966 – Systematisk Botanik. Alger. *In* T. W. Böcher, Morten Lange and Thorvald Sørensen, Botanik 2 (2), Ed. 2, 180 p., København.

Copeland, H. F. 1956 – The classification of lower organisms. Pacific Books, Palo Alto, California. 302 p.

Dillon, L. S. 1963 – A reclassification of the major groups of organisms based upon comparative cytology. Systematic Zool. 12: 71-82.

Dobell, C. C. 1911 – The principle of protistology. Arch. Protistenk. 23: 269–310.

Dougherty, E. C. and Allen, M. B. 1960 – Is pigmentation a clue to protistan phylogeny? *In* Allen, M. B., ed., Comparative Biochemistry of Photoreactive Systems. Symp. Comp. Biol. 1: 129–144. Academic Press, New York.

Hutner, S. H. and Provasoli, L. 1951 – The Phytoflagellates. *In* Lwoff, A., ed., Biochemistry and Physiology of Protozoa 1: 27-128. Academic Press, New York.

Honigberg, B. M., Balamuth, W., Bovee, E. C., Corliss, J. O., Gojdics, M., Hall, R. P., Kudo, R. R., Levine, N. D., Loeblich, A. R., Jr., Weiser, J. and Wenrich, D. H. 1964 – A revised classification of the Phylum Protozoa. Jour. Protozool. 11: 7-20.

Margulis, L. 1969 – New phylogenies of the lower organisms: possible relation to organic deposits in Precambrian sediment: Jour. Geol. 77: 606-617.

Margulis, L. 1971 – The origin of plant and animal cells. Amer. Scientist 59: 230–235.

Papenfuss, G. F., 1955 – Classification of the Algae. *In* A Century of progress in the

natural sciences, 1853-1953, p. 115-224. Calif. Acad. Sci., San Francisco.
PASCHER, A. 1914 – Ueber Flagellaten und Algen. Ber. Deutsch. Bot. Ges. 32: 136-160.
SAGAN, L. 1967 – On the origin of mitosing cells. J. Theoret. Biol. 14: 225-274.
TAPPAN, H. and LOEBLICH, A. R., Jr. 1972 – Fluctuating rates of protistan evolution, diversification and extinction. Internat. Geol. Congr., 24th sess., Montreal, Sec. 7: 205-213.
TAX, S. and CALLENDER, C., Eds. 1960 – Evolution after Darwin. 3. Issues in evolution. Univ. Chicago Press. 310 p.
TILDEN, J. E. 1933 – A classification of the algae based on evolutionary development, with special reference to pigmentation. Bot. Gaz. 95: 59-77.
WHITTAKER, R. H. 1959 – On the broad classification of organisms. Quart. Rev. Biol. 34: 210-226.
WHITTAKER, R. H. 1969 – New concepts of kingdoms of organisms. Science 163: 150-160.